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Inoculation of Loblolly Pine Seedlings at Planting with Basidiospores of Ectomycorrhizal Fungi in Chip Form

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Abstract

Basidiospores of the ectomycorrhizae-forming fungi *Pisolithus tinctorius* and *Scleroderma auranteum* incorporated into an organic hydrocolloid can be used successfully in field inoculation. Containerized loblolly pine seedlings were inoculated during outplanting by this method. This study showed that basidiospore chips were effective inocula in this investigation.
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The symbiotic relationship between the plant's feeder root and a beneficial fungus (mycorrhiza) is an important biological association used in modern plant propagation schemes. In many cases, mycorrhizal plants can be outplanted with the assurance that they will survive and grow better than nonmycorrhizal plants. Research supporting these benefits is well documented (Hacskaylo and Tompkins 1973; Schenck 1982).

Tree seedlings, grown in containers in greenhouses or as bareroot stock in nurseries, can be inoculated with various mycorrhizal fungi at the time of planting to produce mycorrhizal seedlings. There are more than 50 publications on this subject (Riffle and Maronek 1982). The combination of tree species, fungal species, and site should be properly identified and matched for particular plantings to maximize regeneration success in forestry. Seedling growers do not necessarily produce mycorrhizal seedlings intentionally; however, when they do, two major problems may be present: (a) the matched partnership of tree and fungus may not be suitable for the proposed planting site, and (b) the number of mycorrhizae per seedling root system may be below an optimum threshold for tree benefit (Marx et al. 1979).

Inoculating seedlings during the time of outplanting may be a useful alternative if mycorrhizal seedlings are unavailable or too expensive to produce or purchase. This would be analogous to spot fertilizing with nutrient tablets or pellets during planting at particular sites. Therefore, providing a suitable, viable, concentrated source of fungal inoculum next to the seedling's roots may be especially useful if the indigenous fungal propagules are low in density or unable to form suitable mycorrhizae. Although Menge and Timmer (1982) reviewed research concerning the field inoculation of plants by endomycorrhizal fungal inocula, there has been little work on field inoculation of tree seedlings with ectomycorrhizal fungal inocula at the time of forestation. This study[1] was implemented to test field inoculation of containerized loblolly pine (*Pinus taeda* L.) seedlings with the ectomycorrhizal fungi *Pisolithus tinctorius* (Pers.) Coker and Couch and *Scleroderma auranteum* (Pers.) in the form of basidiospore chips, and to determine if ectomycorrhiza formation would enhance the survival and growth of the seedlings.

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Materials and Methods

Loblolly pine seeds were stratified and planted in Spencer-Lemaire "Hillson Rootrainers"[2] during the spring of 1979. Each rootrainer contained four 145 cm^3 cavities. Seedlings were grown in a sterilized mix of equal volumes of No. 3 grade vermiculite, composted sewage sludge, and pine bark. This medium was used to deter infection by natural fungal species during greenhouse production (Reid and Hacskaylo 1982). The following spring (1980), before outplanting, 20 seedlings were selected at random and examined for ectomycorrhizae formed from naturally occurring spores during greenhouse production.

Basidiospores of *Pisolithus tinctorius* (Pt) and *Scleroderma auranteum* (Sa) were collected weekly from puffballs growing on an unreclaimed coal surface mine spoil in western Maryland during the late summer of

[2]The use of trade, firm, or corporation names in this paper is for the information and convenience of the reader. Such use does not constitute an official endorsement or approval by the U.S. Department of Agriculture or the Forest Service or the Maryland Agricultural Experiment Station of any product or service to the exclusion of others that may be suitable.

1979. The upper portions of nonruptured, drying, maturing puffballs were wiped with 70 percent ethanol and sliced off and air dried several days at 26 °C in large sealed paper sacks. Puffball portions were transferred into large plastic bags where they were crushed and sieved through a 45-μm mesh screen. Basidiospores and puffball tissue debris were stored in vented, clear glass bottles at 5 °C in darkness until the spring of 1980.

Basidiospore chips were prepared by gently and thoroughly blending the following materials in sequence: 50 ml autoclaved peatmoss (20 mesh); 25 ml basidiospores and debris; 50 ml J-tac® (organic hydrocolloid); 1000 ml autoclaved sand; and 50 ml warm distilled water. This pasty blend was rolled out onto a flat metal surface to a thickness of 2 mm and then sliced into 450 squares, each square 2 by 2 cm. The chips were air dried under a supported plastic covering for 24 hours, lifted with a spatula, and stored in tight cardboard boxes at 3 °C until used. Control chips devoid of basidiospores and debris also were prepared.

The field site was an agricultural rototilled Beltsville sandy loam soil with a pH of 5.8, Mg, P_2O_5, and K_2O at 240, 132, and 119 kg/ha, respectively, and a 2 percent slope. The site was located at the University of Maryland Plant Research Farm, Prince Georges County, Maryland. The soil had been treated with methyl bromide (MC-33) at the rate of 295 kg/ha under plastic during the previous fall.

There were three treatment groups: control, inoculation with Pt, and inoculation with Sa. Two hundred and forty-six loblolly pine seedlings were simultaneously field inoculated and outplanted at 1.52 by 1.52-m spacings in a completely randomized design in the spring of 1980. Seedlings were planted with a dibble and basidiospore chips were placed vertically 2 cm deep against the uphill side of the planting hole. Weeds were controlled mechanically as needed during the study. A pesticide, Cygon®, was applied to the loblolly foliage once in each of three spring seasons for tip-moth control. Seedling survival, height growth, and diameter (2 cm above root collar) were measured after the first, second, and third season in the field.

Fifteen randomly selected seedlings per inoculation treatment were excavated from the field by shovel after the first season for ectomycorrhizal evaluation and measurements of oven-dry weight. Ectomycorrhizal evaluation consisted of: (a) identification of Pt and Sa ectomycorrhizae by texture and color of mantles, (b) recognition of other ectomycorrhizae formed by unidentified fungi, and (c) determination of percent ectomycorrhizal short roots (the number ectomycorrhizal short roots divided by the number ectomycorrhizal and nonectomycorrhizal short roots). Mushrooms and puffballs of both mycorrhizal and saprophytic fungi that developed during all seasons were removed from the soil and discarded.

Results

Twenty greenhouse-grown seedlings sampled before outplanting were about 9.5 cm tall and 2.3 mm in stem diameter. Eleven seedlings were ectomycorrhizal to a minimum extent with an unidentified tan fungus. Percent ectomycorrhizal short roots of those 11 seedlings ranged from 5 to 30 with an average of 8.2 (average of 4.5 percent ectomycorrhizal short roots for all 20 seedlings).

Ectomycorrhizal condition and dry weight data of an excavated sample of first-season field-grown seedlings from each inoculation treatment are presented in Table 1. All excavated seedlings inoculated with Pt were infected with Pt. Excavated Pt-treated seedlings had an average of 47 percent ectomycorrhizal short roots. Two of the excavated seedlings inoculated with Pt also were infected with an unidentified tan fungus. Seven of fifteen excavated seedlings inoculated with Sa were infected with Sa. Seedlings infected with Sa had an average of 25 percent ectomycorrhizal short roots by Sa. Five of the excavated seedlings inoculated with Sa were infected with an unidentified tan fungus. The ranges of percent of ectomycorrhizal short roots of both Pt and Sa seedlings groups were similar, 5 to 95 and 5 to 85, respectively. Six of fifteen excavated seedlings of the control inoculation sample were ectomycorrhizal with either an unknown rosey-white or tan fungus, but no Pt or Sa was found on control seedlings. These six control seedlings had an average of 16 percent ectomycorrhizal short roots. The average percent ectomycorrhizal short roots for all excavated seedlings formed by both treatment fungi and unknown fungi were 48, 16, and 4, for Pt, Sa, and control seedling groups, respectively. Average root and top dry weights were not significantly different among excavated seedlings for Pt, Sa, and control inoculation treatments after one season of growth (Table 1).

First, second, and third season survival and growth data for all field-grown seedlings are presented in Table 2. Survival of the seedlings inoculated with Sa (96 percent) was significantly greater than that of seedlings inoculated with control chips (88 percent), but not significantly different from that of seedlings inoculated with Pt (92 percent). There were no significant differences in height or diameter growth among seedling inoculation treatment groups in any season.

Numerous puffballs of Pt were observed scattered throughout the study area in the late summer of all seasons. Several Sa puffballs were observed in the late summer of the second and third season. Several *Thelephora terrestris* Ehrh. ex. Fr. basidiocarps were observed in all three seasons.

Discussion

Successful ectomycorrhizal formation of loblolly pine seedlings in which nursery seedbeds were inoculated with basidiospores of Pt has been reported (Marx et al. 1979). Beckjord (unpublished data) successfully inoculated 1-0 bareroot Virginia pine (*Pinus virginiana* L.)

Table 1.—Ectomycorrhizal condition and dry weight data for sample[1] of excavated loblolly pine seedlings inoculated with basidiospore chips of ectomycorrhizal fungi and control chips after one season in the field

Treatment	Number of ectomycorrhizal seedlings infected with—			Percent ectomycorrhizal short roots[2]			Average dry weight all seedlings[2]	
	Any fungus	Treatment fungus	Uniden-tified fungi	Range by treatment fungus	Averages for treatment fungus infected seedlings only	Averages for all seedlings regardless of fungi	Root	Top
							Grams	
Pt	15	15	2	5-95	47	48[a]	46	133
Sa	8	7	5	5-85	25	16[b]	54	144
Control	6	0	6	5-40[3]	16[3]	4[b]	45	112

[1]15 seedlings per inoculation treatment.
[2]Averages in a column with a common superscript or no superscripts are not significantly different at the 0.05 probability level using least significance difference.
[3]Data for seedlings with unknown fungi since no treatment fungus was supplied.

Table 2.—Survival[1] and averages[2] for first, second, and third season field growth of loblolly pine seedlings inoculated with basidiospore chips of ectomycorrhizal fungi and control chips

Treatment	First season		Second season		Third season		Survival end of third season[3]
	Height	Diameter	Height	Diameter	Height	Diameter	
	cm						*Percent*
Pt	52	1.7	137	4.7	226	5.3	92[ab]
Sa	52	1.8	138	4.9	221	5.3	96[a]
Control	55	1.7	139	4.8	226	5.3	88[b]

[1]Chi-square P = 0.05; no change in survival between first, second, and third seasons.
[2]No significant differences at the 0.05 probability level in any column by Duncan's multiple-range test.
[3]Third season survival because there was no change in survival during study period within all treatments.

seedlings planted in an unsterilized field with basidiospore chips of Pt and Sa and Beckjord and McIntosh (1984) successfully inoculated containerized northern red oak (*Quercus rubra* L.) seedlings with basidiospore chips of Pt and Sa on unsterilized field and surface mine soils. In these two studies, ectomycorrhizal formation by Pt and Sa was abundant after the first year in the immediate vicinity where the basidiospore chips were placed. Basidiospore chips were effective inocula in this investigation as well.

All of the excavated seedlings inoculated with Pt were ectomycorrhizal with Pt, and 47 percent of the excavated seedlings inoculated with Sa were ectomycorrhizal with Sa after one growing season. Inoculations in the field with basidiospore chips were effective in producing ectomycorrhizae and may be practical in future regeneration programs when either containerized or bareroot stock is used. Field inoculations might be favorable if (a) mycorrhizal seedlings are unavailable, (b) the tree/fungus partnership could be matched for a particular site, e.g., surface mine spoils, and (c) propagules of indigenous fungal species now should be low because there is no mother source, or low in numbers or unavailable in the root zone (due to recent soil disturbances or seasonal fluctuations in fungus fruiting). Disadvantages in the use of chip inoculations may be: (a) there are a limited number of fungal species that produce abundant spores in puffballs, (b) spore release from chips depends on soil water movement, quantity, and competitive microbial activity, and (c) additional costs and time are necessary to produce and use chips.

The authors believe the advantages in field inoculations via basidiospore chips or pellets exceed the disadvantages if the inoculations are conducted in carefully prescribed situations. Fungi such as Pt and Sa are aggressive pioneer types. These fungi and others may be considered nurse-fungi. They aid in initial seedling establishment on harsh sites and later become secondary to more sophisticated fungi that move into the young established plantation. Inoculation studies should be conducted with many fungal species, soil types, and site conditions, and with containerized and bareroot seedlings of several tree species to refine planting recommendations.

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